

Expression of Enzymes that Metabolize Medications

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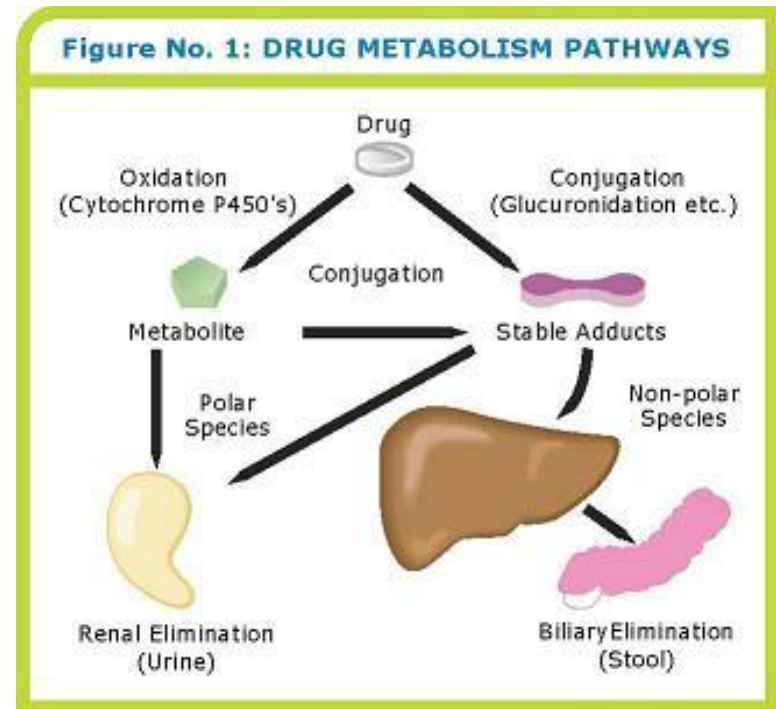
Medications are Metabolized (mostly) in the Liver

Most pharmaceuticals are metabolized by the liver. Clinically-used medication doses are given with normal liver function in mind.

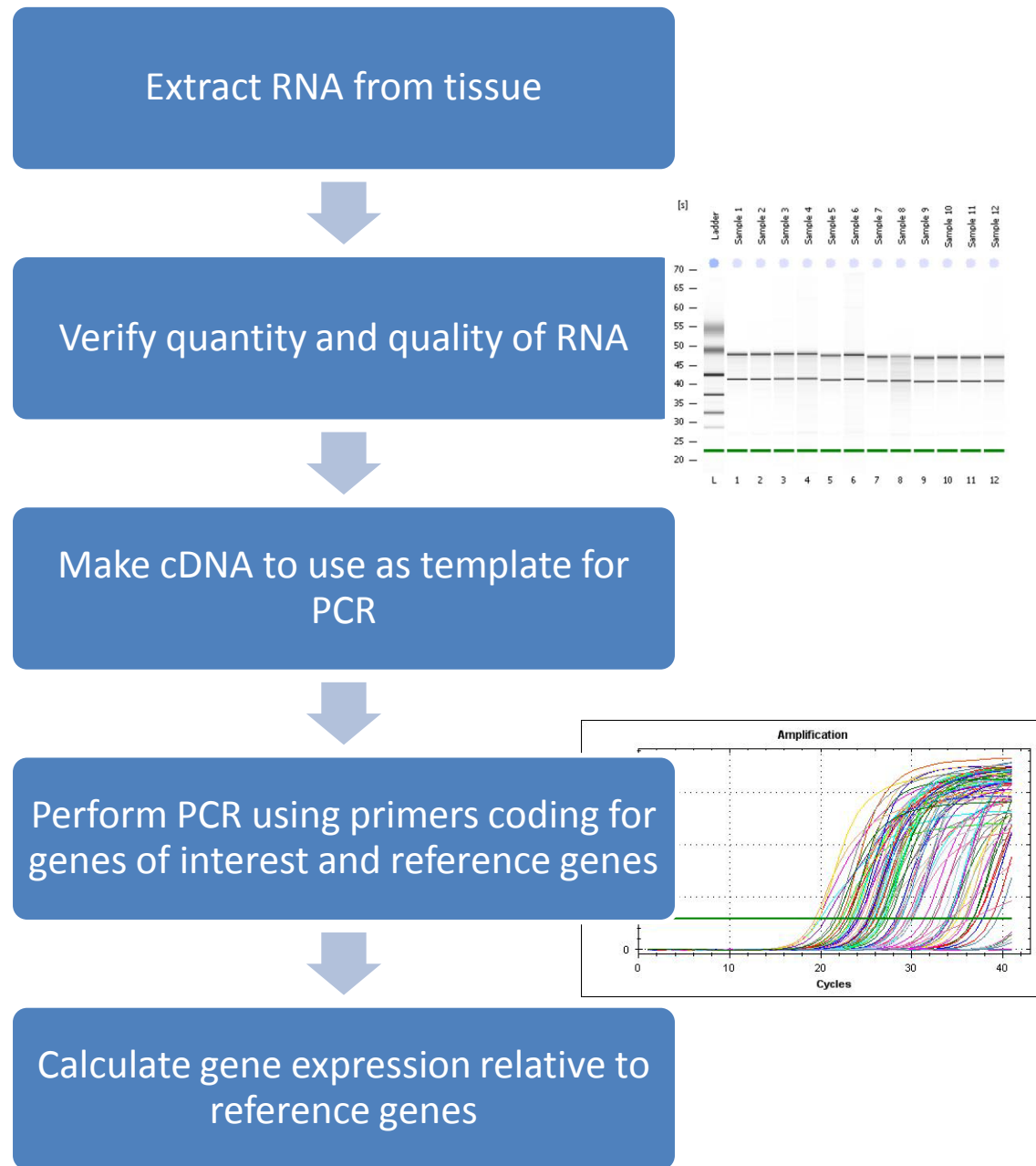
A drug overdose can result if the liver is damaged and removing pharmaceuticals from the circulation at a rate slower than normal.

Alternatively, if liver function is elevated and removing drugs from the system more quickly than usual, it would be as if too little drug had been given for effective treatment.

Because of the importance of the liver in drug metabolism we want to understand the effects of spaceflight on the enzymes of the liver.



Livers were removed from the anesthetized animals immediately after sacrifice, and the livers flash-frozen in liquid nitrogen. Tissue will be homogenized, RNA extracted, and purified. Quality of RNA samples will be evaluated with the Agilent system in the HACD Core Lab. Complementary DNA will be prepared from the RNA samples, and used to run commercially available RT-PCR screening arrays for DNA Repair and Drug Metabolism (SuperArray, SABiosciences, Qiagen). Use of these large pre-optimized primer sets for this pilot experiment will allow efficient screening of over 150 gene products using the BioRad Cfx96 Real-time PCR System in the HACD Core Lab. The results from this phase of the experiment should allow us to not only determine if radiation exposure affects expression level of genes coding for these enzymes, but also to determine if particular groups of enzymes are affected more than others. For example, we might see enzymes that repair breaks in double-stranded DNA upregulated by radiation exposure, while those that repair DNA mismatches or transport drugs across membranes are relatively unaffected, allowing us to focus future research efforts on the most important targets.

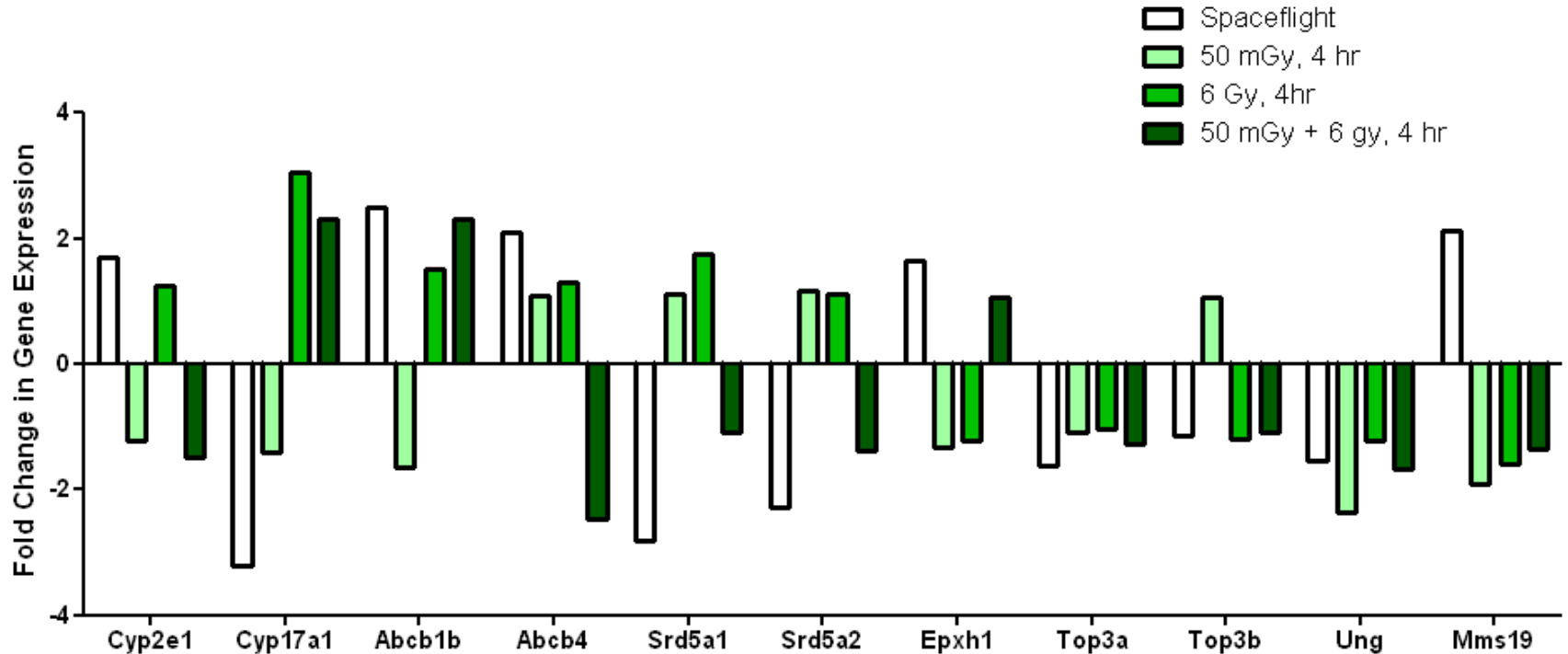


2 test conditions:

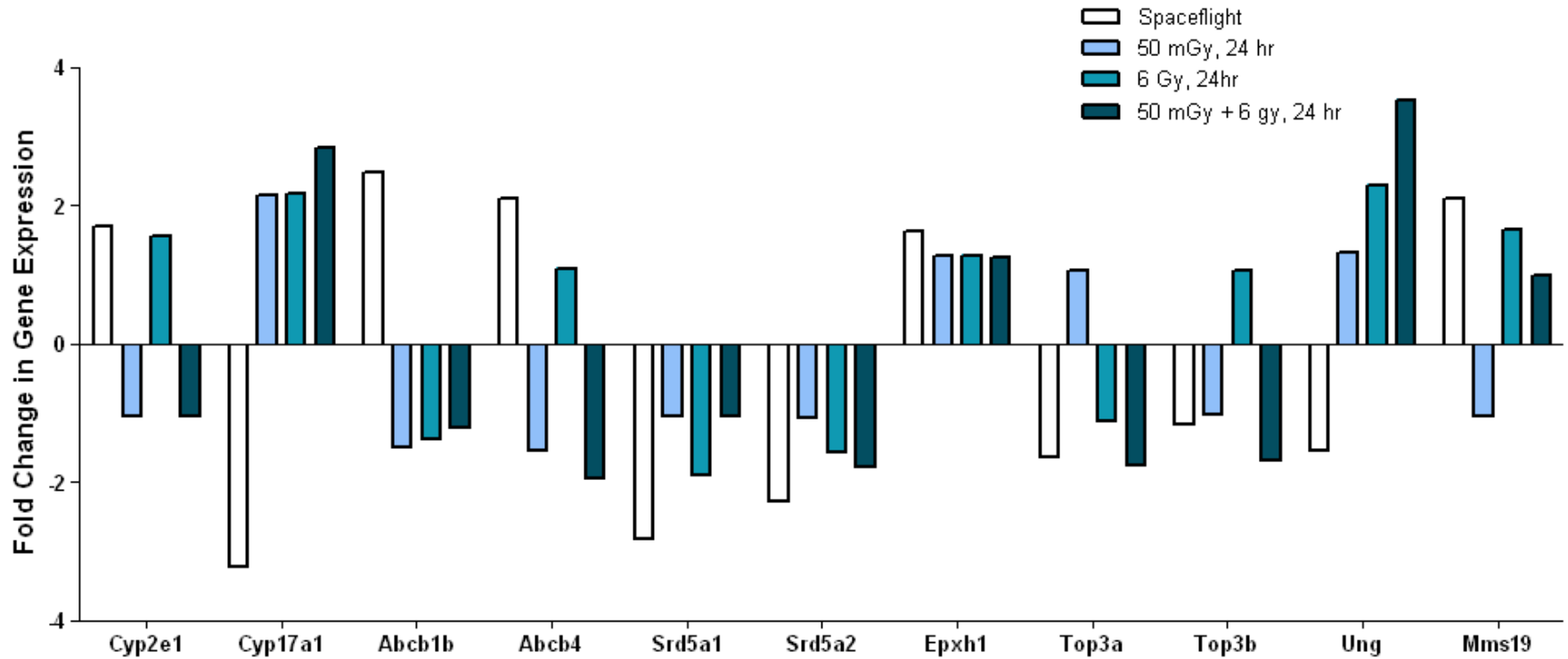
- Spaceflight (mixed radiation and microgravity)
- Chronic exposure (13 days)
- Tissue harvest 3-6 hours after landing

- γ radiation ^{137}Cs , at JSC
- Single, acute exposures of 50 mGy or 6 Gy; double exposure of 50 mGy followed by 6 Gy (24 hours later)
- Tissue harvest at varying time points after exposures: 4 hours later or 24 hours later or 7 days later

How do the radiation exposures compare with spaceflight?

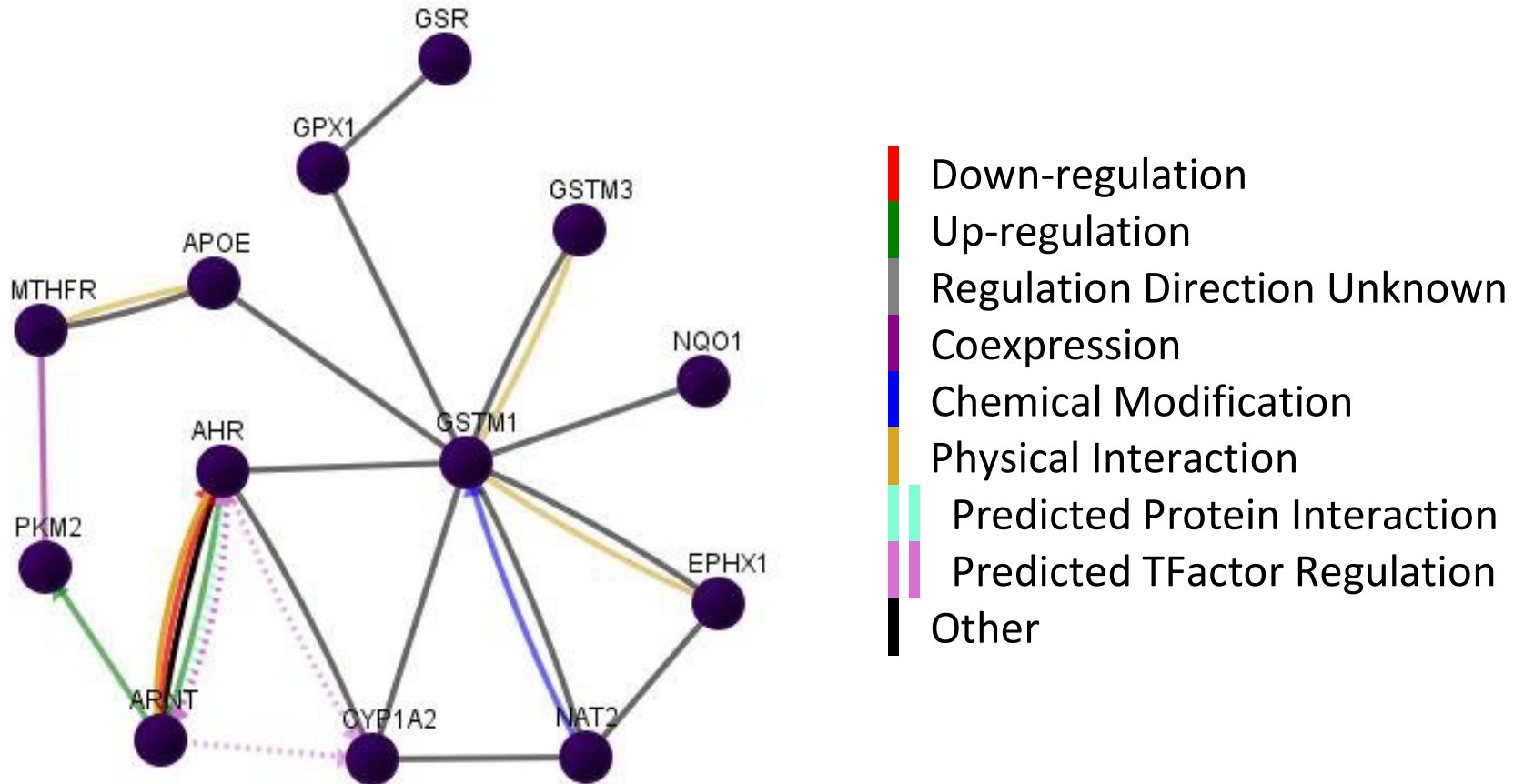


How do the radiation exposures compare with spaceflight?

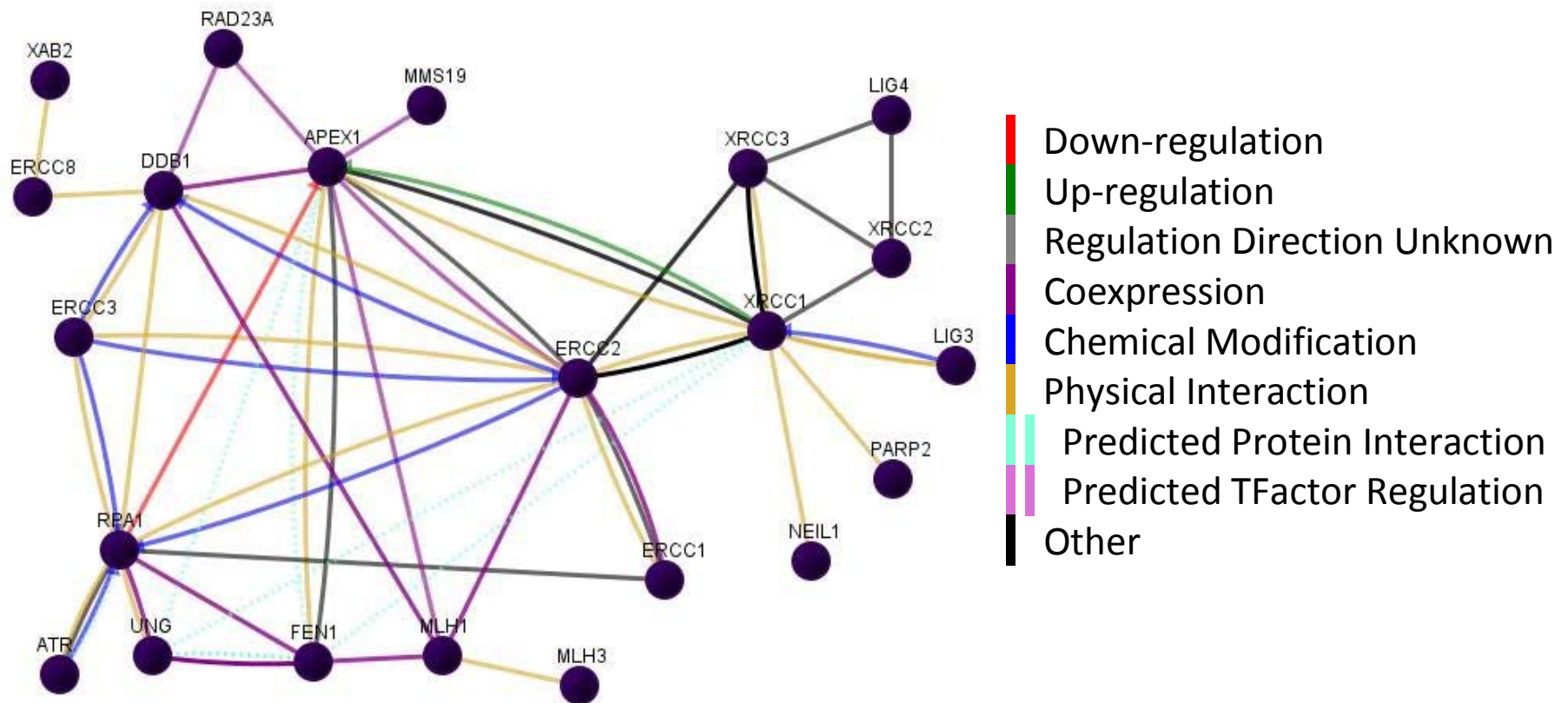


- 168 unique genes tested
- Many were unaffected by any treatment
- Some were upregulated
- Some were downregulated
- Some varied with time after exposure
- Some varied with radiation dose

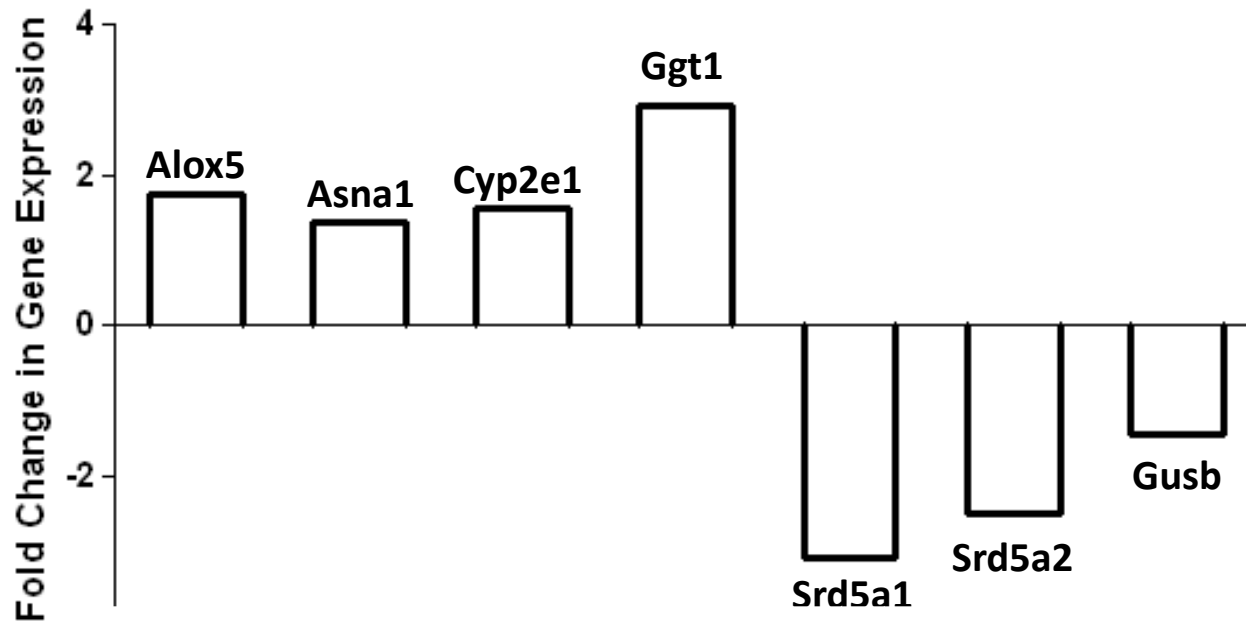
Drug Metabolism Gene Relationships



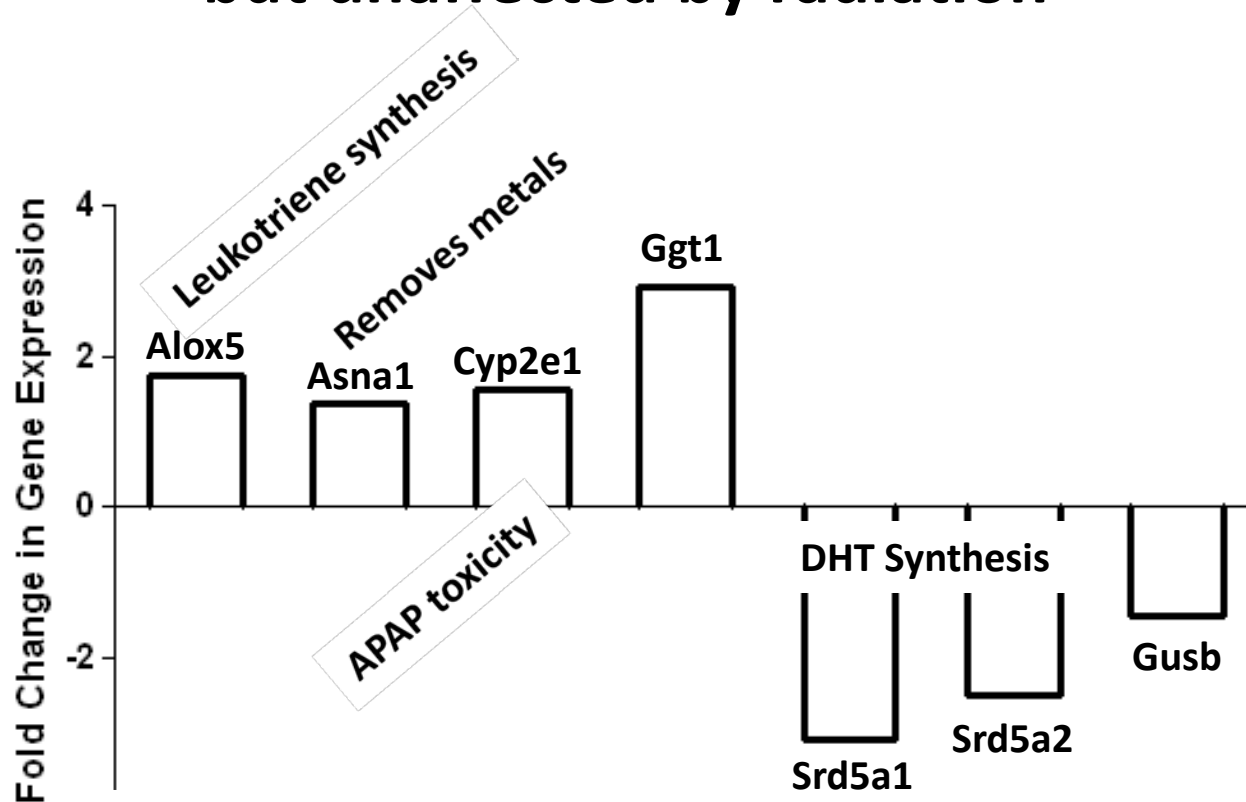
DNA Repair Gene Relationships



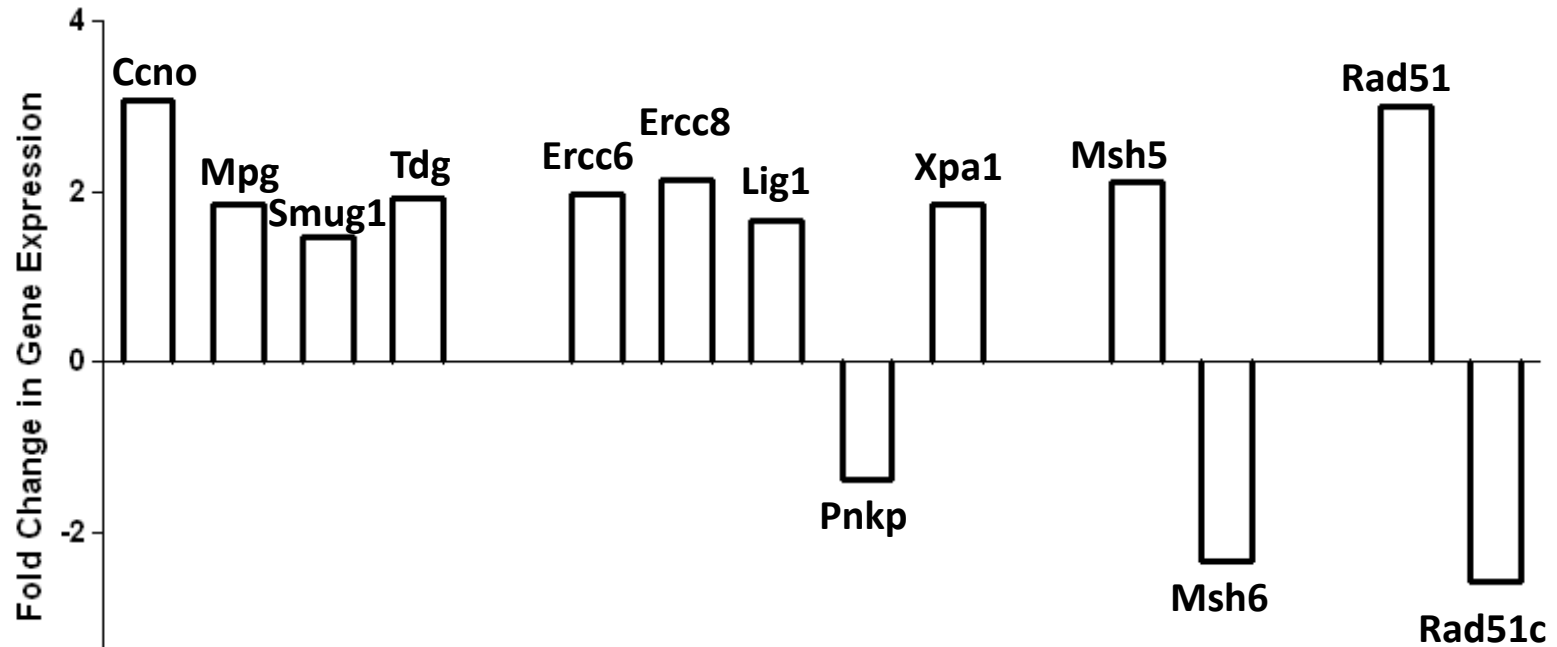
Drug Metabolism Genes Altered by Spaceflight but unaffected by radiation



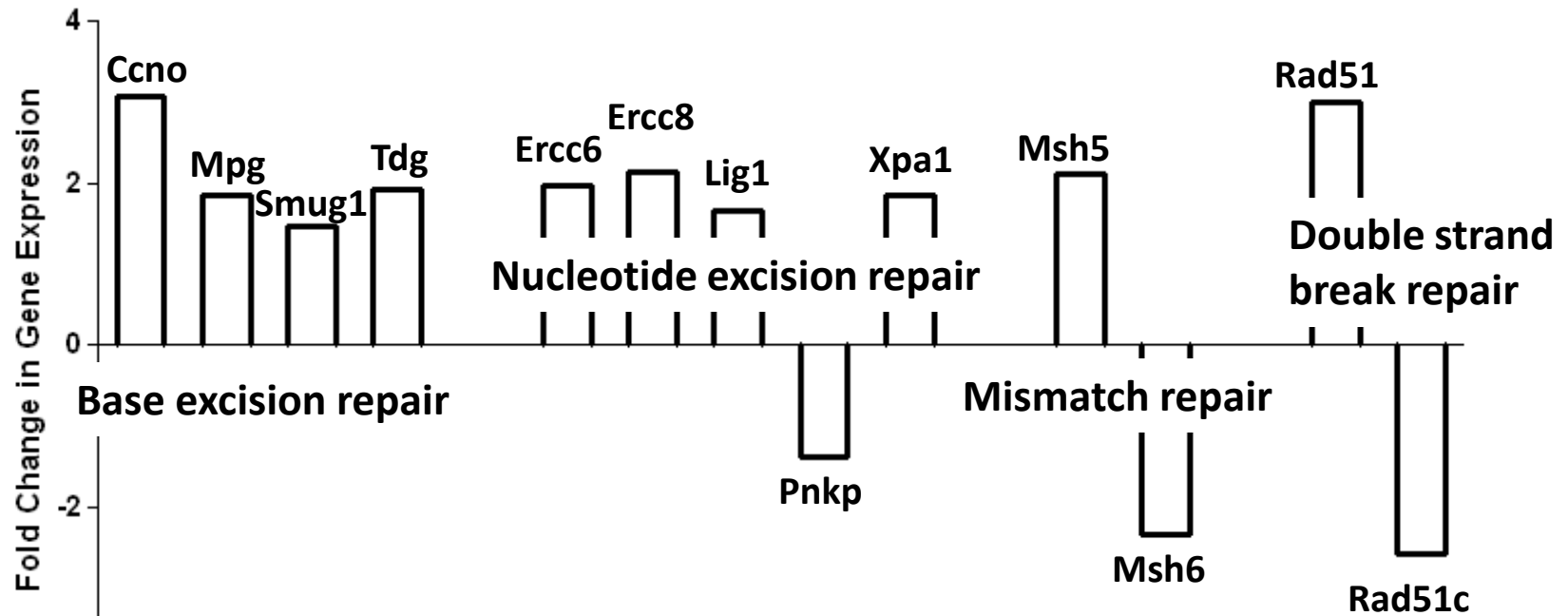
Drug Metabolism Genes Altered by Spaceflight but unaffected by radiation



DNA Repair Genes Altered by Spaceflight but unaffected by radiation



DNA Repair Genes Altered by Spaceflight but unaffected by radiation



Conclusions

- Gene expression was altered by both radiation exposures and by spaceflight.
- There was overlap in the gene expression changes resulting from these different stimuli, but there were also differences.
- The next phase of these experiments will probe for changes in expression and function of the protein products of the genes identified by this qPCR screening method.
- We are beginning to identify specific biochemical pathways that are differentially affected by radiation or by spaceflight, as well as those affected by both.

Acknowledgments

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Technical assistance –

Stephanie Bassett – animal care at JSC

Ye Zhong – Radiation animal experiment team

Lingegowda Mangala - Radiation animal experiment team

Backup Slides

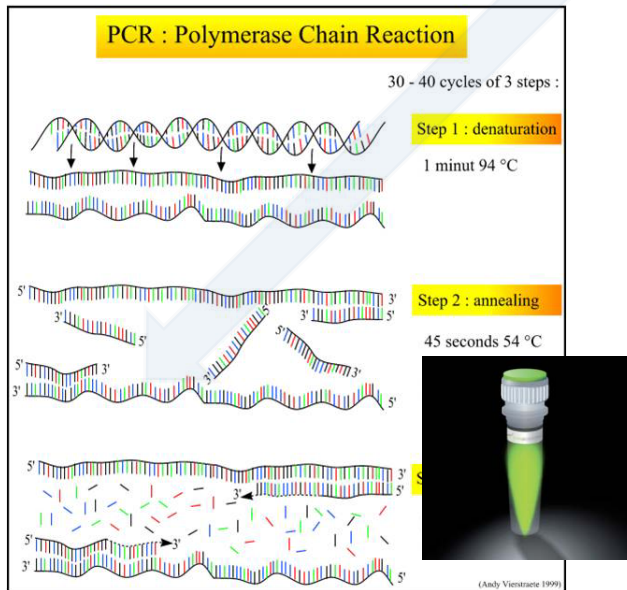
Drugs are metabolized by enzymes, which are proteins.

remember that DNA → RNA → protein

which means that experiments can probe RNA expression levels or protein expression/function

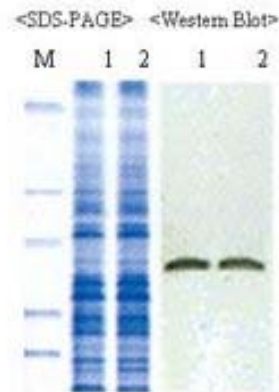
Gene expression

PCR— can examine 96
mRNAs simultaneously



Protein expression

Western blot—
requires unique
antibody for
every protein



Protein function

Enzyme assay —
requires unique
assay for every
enzyme

